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(71)(72) Applicants and Inventors: EVANS, Herbert, J. [US/US]; 906 Hampstead Avenue, Richmond, VA 23226 (US). KINI, R., Manjunatha [IN/SG]; 1 Normanton Park, #15-159P, Singapore 0511 (SG).

(74) Agents: BENT, Stephen, A. et al.; Foley & Lardner, Suite 500, 3000 K Street, N.W., P.O. Box 25696, Washington, DC 20007-8696 (US).

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(57) Abstract

Homologs and analogs of naturally-occurring polypeptides contain one or more interaction sites of the natural counterpart. The interaction sites are flanked by conformation-constraining moieties, such as proline or cysteine.

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POLYPEPTIDES THAT INCLUDE CONFORMATION-CONSTRAINING GROUPS WHICH FLANK A PROTEIN-PROTEIN INTERACTION SITE

Background of the Invention

The present invention relates to improving the bioactivity of a diverse range of peptides and proteins in their various forms (collectively "polypeptides") by employing conformation-constraining residues to flank sites of the polypeptide that are involved in protein-protein interactions.

Protein-protein interactions are crucial to almost every physiological and pharmacological process. These interactions often are characterized by very high affinity, with dissociation constants in the low nanomolar to subpicomolar range. Such strong affinity between proteins is possible when a high level of specificity allows subtle discrimination among closely related structures. The interaction sites of several protein pairs have been identified by strategies such as chemical modification of specific amino acid residues, site-directed mutagenesis, peptide synthesis, X-ray diffraction studies and theoretical approaches.

Certain general structural features have emerged from these studies. For example, some interactions involve more than one interaction site. The phrase "interaction site" is used in this description to denote a site comprised of amino acid residues which is involved in the interaction between two proteins. The high affinities at these interaction sites are attributed to several factors, including shape complementarity, electrostatic and hydrogen bond links, and burial of hydrophobic groups. A protein-protein

interaction may involve one or more of these factors at each interaction site.

The amino acids of an interaction site usually constitute a small proportion of the total amino acids present in the polypeptide. Typically, the number of amino acid residues in a single interaction site ranges from three to six. These residues often are connected by the peptide bonds of adjacent residues in a continuous interaction site. Alternatively, the amino acid residues involved in the interaction are not linked directly by peptide bonds, but rather are brought together by the three-dimensional folding of the protein and are known as "discontinuous" sites. Due to this extensive variability, it has been difficult to identify the amino acids of interaction sites.

The chemical nature of the side chains of the amino acid residues contributes significantly to the interaction, although main chain atoms also can be involved. Positively charged residues (such as lysine, arginine and histidine) can associate through salt bridge links with negatively charged residues (such as aspartic acid and glutamic acid). Additionally, the side chains of leucine, isoleucine, methionine, valine, phenylalanine, tyrosine, tryptophan and proline are often involved in hydrophobic interactions. Precise alignment of atoms between the interaction sites of one protein and its partner also allow multiple Van der Waals interactions and thus increase the likelihood of strong binding between the two interaction partners.

Bernstein *et al.*, *Nature* 340: 482 (1989), proposed a role for methionine in protein-protein interactions, whereby clusters of methionine residues in the 54,000 MW signal recognition particle play a key role in the recognition of signal peptides. Because of the unique flexibility of their side chains, methionine residues located on one face of the surface of the amphiphilic helix provide a malleable, nonpolar surface. It was

postulated that in the binding process, this surface can adapt itself to peptide partners of various dimensions and thus conform to the structure of the signal peptide. A similar mechanism was suggested for the ability of calmodulin to interact with various protein partners. The binding site of calmodulin contains eight exposed methionine residues. Such flexibility of the side chain of methionine might be attributable to the presence of the sulfur atom.

A large number of proteins are synthesized as inactive precursors and activated *in vivo* only where and when they are needed. Accordingly, their activity is strictly regulated so as to contribute to the overall control of physiological processes. Some of these proteins are activated by the action of specific proteinases, and the interaction between the cleavage site and the proteinases should be deemed highly specific. Therefore, the regions around these activation sites form another group of protein-protein interaction sites.

As described above, the diverse properties of the various amino acids affect the characteristics of the interaction site, as well as the polypeptide as a whole. One amino acid residue that has wholly unique structural characteristics is proline.

Proline is the only common imino acid found in proteins. The side chain of proline is bonded to the tertiary nitrogen in a cyclic pyrrolidine ring. This ring inhibits free rotation about the C_α-N bond and thus restricts the range of allowable conformations of the polypeptide backbone. The pyrrolidine ring also constrains the conformation of the adjacent residues. The imino nitrogen of the proline residue lacks a proton that is required for hydrogen bond formation in both the α-helical and ß-pleated sheet conformations. Accordingly, proline is often called a "helix breaker." Additionally, the carbonyl oxygen atom of the amino acid residue immediately preceding proline in the

polypeptide is more electronegative than carbonyl oxygen atoms preceding other amino acid residues. As a result, this carbonyl group has an enhanced tendency to accept and form strong hydrogen bonds.

Proline also differs from other amino acid residues in terms of permissible bond configuration. The partial double bond character of peptide bonds prevents free rotation and can result in either *cis* or *trans* configurations around the peptide bond. In the *cis* configuration, the C_α atoms of adjacent amino acid residues are closer than in the *trans* configuration. This "closeness" often causes steric hindrance between the side chains on the two C_α atoms. Accordingly, almost all peptide bonds are in the *trans* configuration, so that the C_α atoms of adjacent amino acid residues are separated by the greatest distance possible. In contrast to most amino acids, proline residues can more readily assume *cis* configurations because the amide nitrogen is part of a ring. Of course, the ring still imposes conformational constraints by inhibiting free rotation around the α carbons of adjoining residues.

Previous studies have implicated proline residues at some interaction sites of certain classes of molecules. Proline has been thought to be required in the interaction site geometry of a class of proteins known as the serine proteinase inhibitors. This proposal was later retracted because proline was not universally present near interaction sites. See Laskowski and Kato, *Ann. Rev. Biochem.* 49: 593-626 (1980). A proline-directed arginyl cleavage at monobasic processing sites has also been proposed. See Schwartz, *FEBS Letts.* 200: 1-10 (1986). About a third of the monobasic processing sites contain a proline residue either just before or just after the basic residue. A proline residue was also found to be important in the processing of the signal peptide of

human lysozyme. Several proline-directed kinases which phosphorylate their substrates at the residues that are immediately followed by proline residues have been purified from various sources. In some cases, a proline residue two or three residues before the phosphorylation site also appears to have importance. But these phosphorylation sites include only a few examples. Hence, it was assumed heretofore that proline was involved in only a small number of specific cases.

Proline is known to be a helix breaker because it has a secondary amine group, which cannot form hydrogen bonds with neighboring CO groups as other amino acids do. Still, proline is found in some of the surface helices of soluble proteins. The "kink" induced by the proline may help in helical packing by wrapping the helix around a protein core. Recently, the importance of proline residues in transmembrane helices has been noted. Interestingly, the putative transmembrane helices of ion channel peptides have a proline residue within their sequence. These proline residues tend to be conserved among homologous proteins, while similar transmembrane helices of non-transport proteins seem to be devoid of proline residues. The convex side of a proline-containing helix is packed against neighboring transmembrane helices. The unique geometry of the proline frees a non-hydrogen bonded carbonyl oxygen in the helix backbone for binding to a cation.

Because an interaction site of a polypeptide is so difficult to identify, the interaction sites of most proteins have remained unknown. In one of its aspects, the present invention takes advantage of proline positioning to identify interaction sites of proteins. The unique properties of proline have a stabilizing function which, according to another aspect of the present invention, can be used to engineer novel polypeptides based on biologically-active polypeptides. These biologically-active polypeptides possess a

functional activity and include naturally-occurring polypeptides or polypeptides derived therefrom. These novel polypeptides can have improved activities, stabilities or other properties of interest.

Summary of the Invention

It thus is an object of the present invention to provide polypeptides which can mimic or antagonize an activity of biologically-active polypeptide, such as a naturally-occurring polypeptide or a polypeptide derived therefrom.

It is another object to provide polypeptides having conformation-constraining residues which flank one or more interaction sites of the polypeptide.

It is still another object of the present invention to provide polypeptide regions that act as protein-protein interaction sites.

It is also an object of the invention to provide an approach for identifying and synthesizing peptides containing interaction sites.

It is yet another object of the present invention to provide a method for synthesizing polypeptides that are flanked by conformation-constraining moieties.

In accomplishing these and other objects, there have been provided, in accordance with one aspect of the present invention, analogs of biologically-active polypeptides, such as naturally-occurring polypeptides or polypeptides derived therefrom, comprising an interaction site and conformation-constraining moieties flanking the interaction site. The analog typically is shorter than the biologically-active polypeptide, but this need not always be the case. Preferably, the analogs are no more than 30 amino acid residues long, and the conformation-constraining moieties are within 7 amino acid residues of the interaction site. It is also preferred that the conformation-constraining moieties are proline residues.

The analogs can mimic or antagonize an activity of a biologically-active polypeptide. Analogs are provided for that mimic the activity of hypotensive peptides, fibrinolytic peptides, chemotactic peptides, growth promoter peptides, lymphocyte mitogens, immunomodulator peptides, clot-inducing peptides, cardiac stimulant peptides, sweet peptides, taste-modifier peptides, macrophage activating peptides, anti-tumor peptides, Relaxin, platelet aggregation inhibitors, Leech Antiplatelet Protein, Moubatin, and Alzeimer's disease peptides. Analogs are also provided for that antagonize or inhibit the activity of fertility peptides, inflammatory peptides, platelet derived growth factor, blood proteins, and angiotensin II. The inhibited blood proteins include Factor V, Factor VIIa, Factor VIII, Factor IXa, Factor Xa, fibrinogen, prothrombin, von Willebrand Factor and Platelet Glycoprotein IIb.

Analogs are obtainable, pursuant to the present invention, by the steps of identifying an interaction site of a biologically-active polypeptide, such as a naturally-occurring polypeptide or polypeptide derived therefrom, and obtaining a polypeptide that (i) has a different length than the biologically-active polypeptide and (ii) contains the interaction site of the biologically-active polypeptide flanked by conformation-constraining moieties.

In accordance with another aspect of the present invention, there are provided homologs of biologically-active polypeptides, such as naturally-occurring polypeptides or polypeptides derived therefrom, which have at least one interaction site. The homologs comprise most or all of the sequence of the biologically-active polypeptide along with conformation-constraining moieties flanking the interaction site. The conformation-constraining moieties can be placed in biologically-active polypeptides that lack such moieties altogether or

possess such moieties in an undesired location or an undesired form. Preferably, the conformation-constraining moieties comprise proline residues. For example, proline could be used to replace a cysteine that is involved in a disulfide bound when the interaction site is near the cysteine.

The homologs of the present invention can mimic activities of biologically-active polypeptides. In particular, homologs are provided for that mimic the activity of analgesics, appetite suppressants, B-cell differentiating peptides, hypocalcemic agents, hypoglycemic potentiators, hypotensive agents, immune potentiators and somatostatin-like peptides. Additionally, homologs can antagonize or inhibit activities of biologically-active polypeptides. One such homolog is a gastrin-releasing peptide antagonist.

Homologs within the present invention are obtainable by the steps of (i) identifying an interaction site of a biologically-active polypeptide, such as a naturally-occurring polypeptide or polypeptide derived therefrom and (ii) flanking the interaction site with conformation-constraining moieties.

Detailed Description of Preferred Embodiments

It now has been discovered that the biological activity of polypeptides can be enhanced several fold by incorporating proline or other conformation-constraining moieties into regions that flank the interaction site(s) of a given polypeptide. Such enhancement in activity makes it possible to design drugs with greater specificity at decreased cost. Proline residues and other conformation-constraining moieties restrict the number of conformations of the polypeptides to increase the likelihood of the favorable conformation occurring.

The term "polypeptide" is used here to denote all pharmacologically-acceptable forms, such as non-toxic acid or base addition salts.

According to the present invention, an "analog" is a polypeptide containing an interaction site that was obtained or derived from a biologically-active polypeptide, but differs in length from the biologically-active polypeptide upon which it is based. An analog that is shorter than the native polypeptide is referred to as a "truncated analog." In accordance with the present invention, the interaction site(s) of an analog are flanked by conformation-constraining moieties. Typically, these analogs are no more than 30 amino acid residues long, preferably, no longer than 25 amino acid residues and, even more preferably, are no longer than 15 amino acid residues. The conformation-constraining moieties should be within 7 amino acid residues of the interaction site. Preferably, the conformation-constraining moieties are within 4 amino acid residues of the interaction site and, even more preferably, within one amino acid residue of the interaction site. Additionally, the amino acids of the interaction site can be changed, preferably in accordance with the conservative substitutions disclosed herein.

The present invention also is useful for constructing "homologs" of biologically-active polypeptides. A homolog has most or all of the sequence of another, biologically-active polypeptide which contains an interaction site, but the interaction site of the homolog is flanked by conformation-constraining moieties in a manner distinct from the other polypeptide. A homolog thus is a variant formed by placing conformation-constraining moieties adjacent or proximate to the interaction site of the homolog, according to the present invention. This can be done even with polypeptides wherein interaction sites are not flanked with such moieties in the native state.

Accordingly, conformation-constraining moieties can be employed advantageously with all polypeptides having interaction sites, regardless of whether the polypeptide is of natural, recombinant or synthetic origin. The conformation-constraining moieties employed should be sufficiently adjacent or proximate to the interaction site to permit the moieties to exert influence on the site. Homologs can have lengths that differ from that of the native polypeptide. That is, the homolog can be longer or shorter than the native polypeptide. For example, the homolog can contain amino acids in addition to those present in the native polypeptide. Finally, the amino acids of the interaction site can be changed, preferably in accordance with the conservative substitutions disclosed herein.

As is apparent from the above discussion, the concepts implicated by the terms "analog," "truncated analog" and "homolog" are not mutually exclusive. For example, a homolog according to the present invention could comprise a polypeptide where prolines are inserted in the polypeptide sequence. As stated above, a polypeptide modified in this way can also have amino acids removed from the sequence. Thus, a homolog can be shortened so that its length is less than that of the native polypeptide. Other modifications will become apparent to the skilled artisan in view of the present specification.

The present invention employs to advantage the unique structures and characteristics of proline. In proteins, proline residues often affect the conformation of protein-protein interaction sites by breaking the continuity of the adjacent secondary structures, such as α-helices. Small polypeptides often do not have secondary structures, however. Nevertheless, the presence of proline residues in both large and small peptides is useful, pursuant to the present invention, both for locating the interaction

sites of these polypeptide and for stabilizing interaction regions.

The present invention thus encompasses a method for altering or stabilizing the reactivity of interaction sites for bioactivity, by synthesizing a sequence of amino acids where (i) a part of the sequence comprises an interaction site, (ii) the interaction site is flanked on both sides by sequences that contain a proline residue or other conformation-constraining moiety, and (iii) each such moiety is located sufficiently near an interaction site to exert influence over the site. In accordance with the present invention, sequences as thus described can be placed adjacent or proximate to an interaction site on a polypeptide to alter or stabilize the specific reactivity of the site. Such a site can be referred to as being "flanked" or "bracketed" by the conformation-constraining moiety. In this specification, a conformation-constraining moiety so inserted is often referred to as a "bracket."

The present invention also relates to the identification of interaction sites in polypeptides. The interaction site of a polypeptide can be ascertained by searching for flanking proline residues or other conformation-constraining moieties, such as cysteine. For instance, a peptide region that is flanked by two proline residues, a proline residue and a cysteine residue or two cysteine residues is at least a putative interaction site. Typically, the regions that are flanked by these residues comprise fifteen or fewer amino acids.

Via methodology within the present invention, it is possible to produce novel, multifunctional polypeptides, or polypeptides with new functional properties, by inclusion of interaction sites with proline or other conformation-constraining brackets into the polypeptide. The polypeptides of the present

invention can be administered in various non-toxic forms, such as acid or base addition salts.

The inclusion of proline or other constraining brackets allows the interaction site to be altered, which permits targeting of the polypeptide to certain interaction partners found on specific cell or tissue types. Targeting of polypeptide drugs to a specific type of cell or tissue would result in considerable reduction of both the effective dose and the likelihood of side effects. Polypeptides can be custom designed, in accordance with the present invention, to flank an interaction site with brackets to alter or otherwise affect the flanked site.

It is often desirable to insert alanine residues adjacent to the proline brackets. That is, the alanine residues would flank the proline-bracketed interaction sites. The alanine residues serve to protect the amino- and carboxy-terminal ends of the polypeptide.

Several polypeptides having specific, desired activity have been identified. Polypeptides of the structures described here can be synthesized routinely, using solid-phase or solution-phase peptide synthesis. The final peptide preparation can be purified using various chromatographic methods including high performance liquid chromatography and adsorption chromatography. The purity and the quality of the peptides can be confirmed by amino acid analyses, molecular weight determination, sequence determination and mass spectrometry.

The analogs and homologs of the present invention can be combined with a variety of carriers. Pharmaceutically-acceptable carriers include aqueous solutions, non-toxic excipients, including salts, preservatives, buffers and the like, as described in REMINGTON'S PHARMACEUTICAL SCIENCES, 15th Ed. Easton: Mack Publishing Co. pp 1405-1412 and 1461-1487 (1975) and THE NATIONAL FORMULARY XIV., 14th Ed. Washington: American Pharmaceutical Association (1975), the

contents of which are hereby incorporated by reference. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oil and injectable organic esters such as ethyloleate. Aqueous carriers include water, alcoholic/aqueous solutions, saline solutions, parenteral vehicles such as sodium chloride, Ringer's dextrose, etc. Intravenous vehicles include fluid and nutrient replenishers. Preservatives include antimicrobials, anti-oxidants, chelating agents and inert gases. The pH and exact concentration of the various components of the binding composition are adjusted according to routine skills in the art. *See* GOODMAN AND GILMAN'S THE PHARMACOLOGICAL BASIS FOR THERAPEUTICS (7th ed.).

Structural Modifications

Protein interaction sites in great diversity have been identified via the present invention and are described in greater detail below. These interaction sites and surrounding sequences can be altered further in view of the substitution considerations described below.

Conservative substitutions — Amino acids having similar properties can be employed to make conservative substitutions in the sequence of a polypeptide. Such substitutions can help in retaining or, in some cases, enhancing biological properties of the polypeptides. The replacement of one amino acid residue by another residue of the same group are considered conservative substitutions, as set forth below:

group (a) – Lys, Arg, Homoarg and Orn;
group (b) – Leu, Ile, Val, Met and Norleu;
group (c) – Tyr, Phe and Trp;
group (d) – Glu and Asp;
group (e) – Gln and Asn;
group (f) – Ser and Thr; and
group (g) – Ala and Gly.

The abbreviations are as follows: Ala = alanine; Arg = arginine; Asn = asparagine; Asp = aspartic acid; Gln = glutamine; Glu = glutamic acid; Gly = glycine; His = histidine; Homoarg = homoarginine; Ile = isoleucine; Leu = leucine; Lys = lysine; Met = methionine; Norleu = norleucine; Orn = ornithine; Phe = phenylalanine; Pro = proline; Ser = serine; Thr = threonine; Trp = tryptophan; Tyr = tyrosine and Val = valine.

It must be noted, however, that the greater the number of substitutions made in the interaction site, the less predictable its activity will be. Generally, it is preferred to make no more than two amino acid substitutions in the sequence of a given interaction site. In some biologically-active polypeptides, both proline residues and disulfide bridges serve to constrain the conformation of interaction sites. A naturally-occurring interaction site may be bracketed by (1) two proline residues, (2) a proline residue and a cysteine residue (in a disulfide linkage) or (3) two cysteine residues in disulfide linkage, either in linkage with each other or with other residues in the polypeptide, as appropriate.

In most of the polypeptides structures presented here, the proline residues are employed as non-cyclic structural constraints. This means that the constraining proline brackets are only bound to other amino acids by the peptide bond. The present invention also comprehends other non-cyclic structural constraints, such as L-N-methylated amino acid residues or spirolactams. These moieties can be introduced into the peptide backbone. Additionally, side chains can be cyclized to the backbone so as create a L-γ-lactam moiety on each side of the interaction site. See, generally, Hruby *et al.*, "Applications of Synthetic Peptides," *in* SYNTHETIC PEPTIDES: A USER'S GUIDE 259-345 (W.H. Freeman & Co. 1992). Cyclization also can be achieved, for example, by formation of cystine bridges, coupling of amino and carboxy terminal groups of

respective terminal amino acids, or coupling of the amino group of a Lys residue or a related homolog with a carboxy group of Asp, Glu or a related homolog. Coupling of the α-amino group of a polypeptide with the ε-amino group of a lysine residue, using iodoacetic anhydride, can be also undertaken. See Wood and Wetzel, *Int'l J. Peptide Protein Res.* 39: 533-39 (1992).

The conformational restraints imposed by cyclization arise from covalent cross-linking may reduce flexibility too much and even result in strain at the interaction site, which could lead to a loss of function. Proline brackets, on the other hand, allow for some flexibility without causing significant strain at the interaction site. Accordingly, proline is preferred for use in the present invention.

A key aspect of the present invention is the recognition that smaller polypeptides show a considerable amount of flexibility and, consequently, can exist in solution in a very high number of conformers, generated by rotation around all of the N-C_α and C_α-C bonds of the peptide backbone. Pursuant to the present invention, the bracketing of an interaction site by either L- or D-proline imposes constraints on the polypeptide, thereby reducing the number of possible conformers and increasing the relative population that has the favored, active conformation. The introduction of proline brackets to alter or stabilize bioactivity at interaction sites can potentiate the specific action of drugs and other biologically-active agents.

Synthesis of Peptides

Polypeptides within the present invention can be generated directly from the native polypeptides by chemical cleavage, by proteolytic enzyme digestion, and by combinations thereof. Additionally, such polypeptides can be created by synthetic techniques or

recombinant techniques which employ genomic or cDNA cloning methods.

For example, methods of synthesizing polypeptides directly from amino acid derivatives are widely known. Such synthesis can be undertaken with automated peptide synthesizers. Peptides of the structures given below can be routinely synthesized using solid phase or solution phase peptide synthesis.

Site-specific and region-directed mutagenesis techniques also can be employed. See CURRENT PROTOCOLS IN MOLECULAR BIOLOGY vol. 1, ch. 8 (Ausubel *et al.* eds., J. Wiley & Sons 1989 & Supp. 1990-93); PROTEIN ENGINEERING (Oxender & Fox eds., A. Liss, Inc. 1987). In addition, linker-scanning and polymerase chain reaction ("PCR") mediated techniques can be used for purposes of mutagenesis. See PCR TECHNOLOGY (Erlich ed., Stockton Press 1989); CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, vols. 1 & 2, *loc. cit.*

The final peptide preparation can be purified using various chromatographic methods including high performance liquid chromatography and adsorption chromatography. The purity and the quality of the peptides can be confirmed by amino acid analyses, mass spectrometry, molecular weight determination and sequence determination.

Polypeptides within the present invention can be administered in the manner that natural peptides are administered. The method of administration will depend on the site at which the reaction is to occur, as well as the desired result.

The present invention is further illustrated by the following examples. These examples concern the interaction sites of various types of proteins, and are provided to give further insights into the invention. These examples do not limit the scope of the invention.

EXAMPLE I. – EFFECTS OF PROLINE ON CELLULAR ADHESION PROTEINS

Cellular adhesive interactions are involved in tissue development, hemostasis, tumor cell metastasis, intercellular communication, and host defense mechanisms of multicellular organisms. The recognition of extracellular ligands by cell surface receptors is a common but mandatory step in such interactions.

Most of these interactions are mediated by a family of closely related adhesive receptors and have therapeutic implications. For example, the development of antiplatelet drugs is important in the prevention and treatment of atherosclerosis, myocardial infarction, stroke and cancer. The polypeptides involved in platelet aggregation and other adhesive interactions are structurally and immunologically related, and platelet aggregation, one of the specialized adhesive reactions, is easy to monitor by conventional techniques.

Several recognition sequences are involved in the adhesive interactions. The Arg-Gly-Asp (RGD) (SEQ ID NO: 1) tripeptide is a common molecular recognition site implicated in several of these interactions. But the presence of the RGD sequence alone does not necessarily result in the participation of the proteins in adhesive reactions. It appears that the presence of other amino acid residues around the RGD sequence may be important for the presentation of this site. Most adhesive proteins contain at least one proline residue around the RGD sequence, one notable exception being fibrinogen.

Other classes of proteins, such as the disintegrins, possess the RGD tripeptide. Disintegrins are a family of very potent platelet aggregation inhibitors isolated from venoms. These proteins interfere in the interaction between fibrinogen and the glycoprotein IIb-IIIa complex. The RGD sequence in disintegrins is located at the tip of a loop and is

accessible for interaction. Several disintegrins and related inhibitors also contain proline residues.

The effectiveness of proline brackets was demonstrated by constructing several small RGD peptides. Small peptides containing the RGD sequence inhibit adhesive reactions, including platelet aggregation. The sequence Ile-Ala-Arg-Gly-Asp-Met-Asn-Ala was selected as typical of peptides containing the Arg-Gly-Asp sequence. Proline residues were substituted on one or both sides of the Arg-Gly-Asp-Met sequence. Four peptides, Ile-Ala-Arg-Gly-Asp-Met-Asn-Ala (P-1) (SEQ ID NO: 2), Ile-Pro-Arg-Gly-Asp-Met-Asn-Ala (P-2) (SEQ ID NO: 3), Ile-Ala-Arg-Gly-Asp-Met-Pro-Ala (P-3) (SEQ ID NO: 4), and Ile-Pro-Arg-Gly-Asp-Met-Pro-Ala (P-4) (SEQ ID NO: 5), were synthesized by solid phase peptide synthesis. After extraction, the peptides were purified by a reverse phase HPLC system to more than 95% purity, with yields between 80% and 90%. The structures of individual peptides were confirmed by amino acid analysis, and their masses were confirmed by fast atom bombardment mass spectra.

The inhibition of platelet aggregation by these peptides was studied in a whole blood aggregometer. Platelet aggregations were initiated by the addition either of collagen or of ADP. All four peptides inhibited platelet aggregation.

To compare the inhibitory potencies, the dose-response relationships were determined for the polypeptides, as identified below in Table 1. The inhibitory potencies of the polypeptides were P-4 > P-3 = P-2 > P-1. The concentration of polypeptides inhibiting platelet aggregation by 50% ("the IC_{50} value") was determined from the dose-response curves; the fold-increase in the inhibitory potencies also was determined (Table 1). The inhibitory potency of Ile-Ala-Arg-Gly-Asp-Met-Asn-Ala is comparable with that of the Arg-Gly-Asp-Ser peptide (SEQ ID NO: 6). Incorporation of proline on either side of Arg-Gly-Asp

enhances the potency to about the same extent. Inclusion of proline residues on both sides enhanced the antiplatelet effect of the Arg-Gly-Asp peptide by 7 to 13-fold.

Table 1

Peptide	Donor 1 IC50	Donor 1 Fold	Donor 2 IC50	Donor 2 Fold
Collagen-induced aggregation				
P-1	84.5	-----	67.3	-----
P-2	48.8	1.73	27.6	2.44
P-3	37.5	2.25	27.6	2.44
P-4	6.4	13.10	8.4	8.01
Arg-Gly-Asp-Ser	57.8	-----	32.3	-----
ADP-induced aggregation				
P-1	27.3	-----	22.5	-----
P-2	21.5	1.27	18.9	1.19
P-3	21.0	1.30	16.7	1.34
P-4	4.0	6.77	2.2	10.27
Arg-Gly-Asp-Ser	29.9	-----	13.8	-----

The inhibitory potency of P-2, P-3, and P-4 were compared with P-1 to obtain the fold increase in its potency.

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There are other RGD-containing peptides that inhibit the interaction between fibrinogen and its platelet receptor, the glycoprotein IIb-IIIa complex, and thus are platelet aggregation inhibitors. These peptides can be administered by intravenous injections, *in situ* injections, local applications, inhalation, oral administration using coated polymers, dermal

patches or other appropriate means, usually in a dosage of 100-2000 nM. Sequences include:

- Ile-Pro-Arg-Gly-Asp-Tyr-Pro-Ala (PYP) (SEQ ID NO: 7)
- Ile-Pro-Arg-Gly-Asp-Phe-Pro-Ala (PFP) (SEQ ID NO: 8)
- Ile-Pro-Arg-Gly-Asp-Trp-Pro-Ala (PWP) (SEQ ID NO: 9)
- Ile-Pro-Lys-Gly-Asp-Trp-Pro-Ala (PKWP) (SEQ ID NO: 10)
- Ile-Pro-Homoarg-Gly-Asp-Trp-Pro-Ala (PhRWP) (SEQ ID NO: 11)

Each of these peptides have the generalized formula b - Pro - a - g - d - b or c - Pro - g based on the conservative substitution groups discussed above.

Another important interaction site on adhesive proteins is the sequence Leu-Asp-Val (SEQ ID NO: 12 wherein proline brackets are provided to form the sequence Ala-Pro-Leu-Asp-Val-Pro-Ala (SEQ ID NO: 13). Additionally, the interaction site having the sequence Val-Thr-Cys-Gly (SEQ ID NO: 14) can be bracketed providing the sequence Ala-Pro-Val-Thr-Cys-Gly-Pro-Ala (SEQ ID NO: 15).

These data demonstrate that the inclusion of conformation-constraining moieties can have desirable effects on an interaction site. These data also demonstrate that interaction sites possess activity when present in a polypeptide that differs from the native form. Finally, these data show the propriety of identifying interaction sites by the presence of proline brackets. Accordingly, the below-described analogs and homologs of the present invention, which contain conformation-constraining brackets like proline, have useful activities.

EXAMPLE II. TRUNCATED ANALOGS

The following sequences are obtained from naturally-occurring polypeptides that contain proline brackets or proline/cysteine brackets. These polypeptides can be shortened to form fragments that contain one or more interaction sites of interest. As stated above, these fragments are referred to as "truncated analogs."

The presence of the proline brackets is useful for identifying the interaction sites of the polypeptides to permit construction of the truncated analogs. The truncated analogs below can be employed in a manner similar to the naturally-occurring polypeptide. In this sense, the truncated analogs mimic the naturally-occurring polypeptide.

Hypotensive Peptides

Applications: Treatment of cardiovascular diseases by reduction of blood pressure.

1. Origin Type: **Calciseptine**

Mechanism: Binds to L-type calcium channels in aorta and cardiac myocytes and inhibits the calcium current. This helps in the relaxation of these muscles and thus reduces blood pressure.

Dose: 60 to 120 μg per rat (5 to 10 μM). Comparable to diltiazem (in Cardizem-CD), which is on the market.

Advantages: Preliminary studies indicate that in the presence of diltiazem there is a small increase in the diastolic pressure. This suggests incomplete relaxation of the heart between beats when treated with diltiazem, which is detrimental. Treatment with the peptide, however, does not increase diastolic pressure. Also the peptide seems to exert anti-arrythmogenic activity.

Administration: Intravenous injections, inhalation, coated polymers (oral), implants, skin patches, and other appropriate means.

Structure:

- Ala-Pro-Thr-Ala-Met-Trp-Pro-Ala (HP-1 or L-Calchin) (SEQ ID NO: 16)

2. Origin Type: **Adrenomedulin**

Mechanism: Reduces the blood pressure in rats through an unknown mechanism, possibly involving nerve terminals.

Dose: 30 to 100 μg/rat (30 to 100 nmole/rat)

Advantages: Increases cyclic AMP in platelets and may thus possess antiplatelet activity. Such antiplatelet activity is beneficial in reducing the risk of myocardial infarction and stroke in individuals with high blood pressure.

Administration: Intravenous injections, inhalation, coated polymers (oral), implants, skin patches and other appropriate means.

Structure:

- Ala-Pro-Arg-Ser-Lys-Ile-Ser-Pro-Gln-Gly (HP-2 or Amulin) (SEQ ID NO: 17)

3. Origin Type: **Maxadilan**

Mechanism: Reduces blood pressure through vasodilation.

Dose: 200-500 nM (10 μg/rat).

Administration: Intravenous injections, inhalation, coated polymers (oral), implants and skin patches.

Structures:

- Gln-Leu-Pro-Gly-Asn-Ser-Val-Phe-Lys-Glu-Pro-Met (HP-3 or Dilamax-1) (SEQ ID NO: 18)
- Phe-Thr-Ser-Met-Asp-Thr-Ser-Gln-Leu-Pro-Gly (HP-4 or Dilamax-2) (SEQ ID NO: 19)

Fibrinolytic Peptides

Application: For dissolving clots formed in various thrombotic and hemostatic ailments including myocardial infarction and stroke.

Mechanism: Binds to plasminogen and non-proteolytically activates plasminogen, which dissolves fibrin clot.

Dose: 1-300 μM.

Administration: Intravenous injections, *in situ* injections, local applications, inhalation, oral administration using coated polymers, dermal patches and other appropriate means.

1. Origin Type: **Staphylokinase**
   Structures:
   - Ser-Pro-Arg-Tyr-Val-Glu-Phe-Pro-Ile-Lys-Pro-Gly (FP-STA1) (SEQ ID NO: 20)
   - Phe-Pro-Ile-Thr-Glu-Lys-Gly-Phe-Val-Val-Pro-Asp (FP-STA2) (SEQ ID NO: 21)
   - Val-Pro-Asp-Leu-Ser-Glu-His-Ile-Lys-Asn-Pro-Gly (FP-STA3) (SEQ ID NO: 22)
   - Lys-Pro-Asp-Asp-Ala-Ser-Tyr-Phe-Glu-Pro-Thr-Gly-Pro-Tyr (FP-STA4) (SEQ ID NO: 23)

2. Origin Type: **Streptokinase**
   Structures:
   - Arg-Pro-Tyr-Lys-Glu-Lys-Pro-Val (FP-SRP1) (SEQ ID NO: 24)
   - Thr-Pro-Leu-Asn-Pro-Asp-Asp-Asp-Phe-Arg-Pro-Gly (FP-SRP2) (SEQ ID NO: 25)
   - Ser-Pro-Lys-Ser-Lys-Pro-Phe-Ala-Thr-Asp-Ser-Gly-Ala-Met-Pro-His (FP-SRP3) (SEQ ID NO: 26)

Chemotactic Peptides

Applications: Attract neutrophils and macrophages and hence will be useful in enhancing body defense mechanism at a required site.

Mechanism: Probably through specific receptor interaction.

Administration: Intravenous injections, *in situ* injections, local applications, inhalation, oral administration using coated polymers, dermal patches and other appropriate means.

1. Origin Type: **CP-10**
   Dose: 10-100 pM
   Structure:
   - Ala-Pro-Gln-Phe-Val-Gln-Asn-Ile-Pro-Ala (CP-CP10A) (SEQ ID NO: 27)

2. Origin Type: **Interleukin-8**
   Dose: 5-100 nM
   Structure:
   - Lys-Glu-Leu-Arg-Pro-Gln (CP-IL8A) (SEQ ID NO: 28)

Origin Type: **α-1 Proteinase Inhibitor**
Dose: 5-100 nM
Structures:
- Ala-Pro-Glu-Val-Lys-Phe-Asn-Lys-Pro-Phe-Val (CP-αPI1) (SEQ ID NO: 29)
- Ser-Pro-Leu-Phe-Ile-Gly-Lys-Val-Val-Asn-Pro-Thr (CP-αPI2) (SEQ ID NO: 30)

Growth Promoter Peptides

Neurite-promoting peptides

Applications: In treatment of injuries to nervous system and trauma. Helpful in promoting growth of neurites to regenerate broken connections caused by injury.

1. Origin Type: **Pleiotrophin**
   Mechanism: Through interaction with specific receptors.
   Dose: 50 to 200 nM.

Advantages: Smaller size of the peptide may help the molecule cross the blood-brain barrier.

Administration: Intravenous injections, *in situ* injections, inhalation, oral administration using coated polymers, dermal patches and other appropriate means.

Structures:

- Ser-Lys-Pro-Ala-Gly-Lys-Leu-Thr-Lys-Ser-Lys-Pro-Gln-Ala (NPP-PT1) (SEQ ID NO: 31)
- Ser-Lys-Pro-Ala-Gly-Lys-Leu-Thr-Lys-Pro-Lys-Pro-Gln-Ala (NPP-PT2) (SEQ ID NO: 32)
- Lys-Ile-Pro-Ala-Asn-Trp-Lys-Lys-Gln-Phe-Pro-Ala (NPP-PT3) (SEQ ID NO: 33)

Homology: The NPP-PT1 and NPP-PT2 polypeptides have the generalized formula f - a - Pro - g - g - a - b - f - a based on the conservative substitution groups discussed above.

2. Origin Type: **Ciliary Neurotrophic Factor**

Mechanism: Probably through specific receptors.

Dose: 5-200 nM.

Advantages: Smaller size of the peptide may help the molecule cross the blood-brain barrier.

Administration: Intravenous injections, *in situ* injections, inhalation, oral administration using coated polymers, dermal patches and other suitable means.

Structures:

- Val-Pro-Val-Ala-Ser-Thr-Asp-Arg-Trp-Ser-Glu-Leu-Thr-Glu-Ala (NPP-CNTF1) (SEQ ID NO: 34)
- Ile-Pro-Arg-Asn-Glu-Ala-Asp-Gly-Met-Pro-Ile (NPP-CNTF2) (SEQ ID NO: 35)

Granulocyte Colony Stimulating Peptides

Applications: Helpful in proliferation and differentiation of hemopoietic precursors and

stimulation of mature cells. For treatment of neutropenia in a variety of clinical situations.

Mechanism: Through interaction with specific receptors.

Dose: 50 to 200 nM.

Administration: Intravenous injections, *in situ* injections, inhalation, oral administration using coated polymers, dermal patches and other appropriate means.

1. Origin Type: **GCSP**

Structures:

- Ala-Pro-Ser-Gln-Ala-Leu-Gln-Leu-Ala-Pro-Ala (GCSP-1) (SEQ ID NO: 36)
- Ala-Pro-Ala-Leu-Gln-Pro-Thr-Gln-Gly-Ala-Met-Pro-Ala (GCSP-2) (SEQ ID NO: 37)
- Ile-Pro-Trp-Ala-Pro-Leu-Ser-Ser-Ala-Pro-Ser (GCSP-3) (SEQ ID NO: 38)
- Ser-Pro-Glu-Leu-Gly-Pro-Thr-Leu (GCSP-4) (SEQ ID NO: 39)
- Thr-Pro-Leu-Gly-Pro-Ala-Ser-Ser-Leu-Pro-Gln-Ser (GCSP-5) (SEQ ID NO: 40)

2. Origin Type: **IL-3**

Structure:

- Leu-Pro-Leu-Ala-Thr-Ala-Ala-Pro-Thr-Arg-His-Pro-Ile (IL3A) (SEQ ID NO: 41)

Progen (SCF Peptides)

Applications: Helpful in proliferation and differentiation of hemopoietic precursors and stimulation of mature cells. For treatment after bone marrow transplants and various other clinical situations.

Origin Type: **Stem Cell Factor**

Mechanism: Interact with specific receptors and enhance the growth and differentiation of progenitor cells.

Dose: 100 to 500 nM.

Administration: Intravenous injections, *in situ* injections, inhalation, oral administration using coated polymers, dermal patches and other suitable means.

Structures:

- Asp-Pro-Val-Val-Ser-Ser-Thr-Leu-Ser-Pro-Glu (SCF-1; Progen-1) (SEQ ID NO: 42)
- Val-Pro-Gly-Met-Asp-Val-Leu-Pro-Ser (SCF-2; Progen-2) (SEQ ID NO: 43)
- Ser-Pro-Glu-Pro-Arg-Leu-Phe-Thr-Pro-Glu (SCF-3; Progen-3) (SEQ ID NO: 44)

Endothelial Growth Peptides

Applications: For inducing growth of vasculature. Helpful in wound healing after surgical procedures as well as in severe damage caused by accidents.

Origin Type: **Vascular Permeability Factor**

Dose: 50 to 300 nM.

Administration: Intravenous injections, *in situ* injections, topical application, inhalation, oral administration using coated polymers, dermal patches or other suitable means.

Structure:

- Tyr-Pro-Asp-Glu-Ile-Glu-Tyr-Ile-Phe-Lys-Pro-Ser (VPF-1) (SEQ ID NO: 45)

Neurotrophic Factor

Applications: For treatment of injuries and trauma to the nervous system. Helpful in promoting growth of neurites to regenerate broken connections caused by injury.

Origin Type: **Glial Cell Line-Derived Neurotrophic Factor**

Mechanism: Promotes the growth of dopaminergic neurons through interaction with specific receptors.

Dose: 50 to 200 nM.

Advantages: The small size of the peptide may help the molecule cross the blood-brain barrier.

Administration: Intravenous injections, *in situ* injections, inhalation, oral administration using coated polymers, dermal patches and other appropriate means.

Structures:

- Ser-Pro-Asp-Lys-Gln-Ala-Ala-Ala-Leu-Pro-Arg-Arg (NPP-GDNF1) (SEQ ID NO: 46)
- Asn-Pro-Glu-Asn-Ser-Arg-Pro-Lys (NPP-GDNF2) (SEQ ID NO: 47)

Lymphocyte Mitogens

Origin Type: ***Streptococcus pyrogenes* Mitogenic Factor**

Dose: 1 to 100 μM.

Administration: Intravenous injections, *in situ* injections, inhalation, oral administration using coated polymers, dermal patches and other appropriate means.

Structures:

- Thr-Pro-Ala-Leu-Phe-Pro-Lys (LM-SP1) (SEQ ID NO: 48)
- Asn-Pro-Ala-Gly-Trp-Thr-Gly-Asn-Pro-Asn (LM-SP2) (SEQ ID NO: 49)
- Ala-Pro-Ile-Tyr-Asn-Ala-Asp-Glu-Leu-Ile-Pro-Arg (LM-SP3) (SEQ ID NO: 50)

Immunomodulator Peptides

1. Origin Type: **Ling-Zhi-8**

Applications: Combating several inflammatory autoimmune diseases and others. Antidiabetic and antitumor effects.

Mechanism: Activate T-cells and facilitate cellular interaction.

Dose: 10-150 nM.

Administration: Intravenous injections, *in situ* injections, inhalation, oral administration using coated polymers, dermal patches and other appropriate means.

Structures:

- Tyr-Thr-Pro-Asn-Trp-Gly-Arg-Gly-Asn-Pro-Asn-Asn (IP-LZ1) (SEQ ID NO: 51)
- Gly-Asn-Pro-Asn-Asn-Phe-Ile-Asp-Thr-Val-Thr-Phe-Pro-Lys-Val (IP-LZ2) (SEQ ID NO: 52)

2. Origin Type: **IL-4**

Mechanism: Bind to specific receptors and inhibit cell-mediated immunity, enhances humoral immunity.

Dose: 10-500 nM.

Administration: Intravenous injections, *in situ* injections, inhalation, oral administration using coated polymers, dermal patches and other appropriate means.

Structures:

- Leu-Pro-Val-Thr-Asp-Ile-Phe-Ala-Ala-Pro-Lys (IP-IL4A) (SEQ ID NO: 53)
- Ala-Pro-Val-Lys-Glu-Ala-Asn-Gln-Pro-Thr (IP-IL4B) (SEQ ID NO: 54)
- Thr-Pro-Ala-Thr-Glu-Leu-Thr-Val-Pro-Asp (IP-IL4C) (SEQ ID NO: 55)
- Ser-Pro-His-Glu-Lys-Asp-Thr-Arg-Pro-Leu (IP-IL4D) (SEQ ID NO: 56)

3. Origin Type: **IL-10**

Mechanism: Bind to specific receptors and inhibit cell-mediated immunity, enhances humoral immunity.

Dose: 10-500 nM.

Administration: Intravenous injections, *in situ* injections, inhalation, oral administration using coated polymers, dermal patches and other appropriate means.

Structures:

- Val-Pro-Gln-Ala-Glu-Asn-Gln-Asp-Pro-Asp-Ile (IP-IL10A) (SEQ ID NO: 57)
- Arg-Pro-His-Arg-Phe-Leu-Pro-Ala (IP-IL10B) (SEQ ID NO: 58)

- His-Phe-Pro-Gly-Asn-Leu-Pro-Asn-Met-Leu (IP-IL10C) (SEQ ID NO: 59)

Clot-inducing Peptides

Applications: Effective in controlling blood loss in various situations, including surgical procedures and accidents.

1. Origin Type: **Staphylocoagulase**

Mechanism: Bind and non-proteolytically activate prothrombin which in turn induces blood clotting.

Dose: 1-200 μM.

Administration: Topical applications by spraying at the site of the damage.

Structures:

- Thr-Pro-Ala-Ile-Asp-Leu-Leu-Glu-Thr-Tyr-Lys-Tyr-Gly-Asp-Pro-Ile (CIP-STA1) (SEQ ID NO: 60)
- Asp-Pro-Ile-Tyr-Lys-Glu-Ala-Lys-Asp-Arg-Leu-Met-Thr-Arg (CIP-STA2) (SEQ ID NO: 61)
- Asn-Pro-His-Lys-Ile-Thr-Asn-Glu-Arg-Ile-Lys (CIP-STA3) (SEQ ID NO: 62)
- Glu-Leu-Arg-Ala-Lys-Leu-Asp-Leu-Ile-Leu-Pro-Asp (CIP-STA4) (SEQ ID NO: 63)
- Ser-Pro-Val-Val-Lys-Glu-Glu-Asn-Lys-Val-Glu-Glu-Pro-Gln-Leu (CIP-STA5) (SEQ ID NO: 64)

2. Origin Type: **Botrocetin**

Mechanism: Interact with von Willebrand factor and/or glycoprotein Ib and induce platelet aggregation.

Dose: 1-200 μM.

Applications: Effective in controlling blood loss in various situations, including surgical procedures and accidents.

Administration: Topical applications by spraying at the site of the damage.

Structures:

- Lys-Pro-Thr-Asn-Asn-Lys-Trp-Trp-Ile-Ile-Pro-Ala (CIP-BCTN1) (SEQ ID NO: 65)

- Ala-Pro-Ser-Gly-Trp-Ser-Ser-Tyr-Glu-Gly-Asn-Pro-Tyr (CIP-BCTN2) (SEQ ID NO: 66)
- Asn-Pro-Phe-Val-Ala-Lys-Ser-Pro-Ala (CIP-BCTN3) (SEQ ID NO: 67)

Cardiac Stimulant Peptides

Origin Type: **Anthopleurin A and B**

Mechanism: Bind to voltage-gated sodium channels and prolong the action potential, which causes cardiostimulatory effects.

Dose: 50-1000 nM.

Administration: Intravenous injections, inhalation, oral administration using coated polymers, dermal patches and other appropriate means.

Structures:

- Arg-Pro-Arg-Gly-Asn-Thr-Leu-Ser-Pro-Ala (CSP-APB1) (SEQ ID NO: 68)
- Gly-Pro-Ser-Val-Arg-Gly-Asn-Thr-Leu-Ser-Pro-Ala (CSP-APA1; Aleurin) (SEQ ID NO: 69)

Homology: The CSP-APA1 and CSP-APB1 polypeptides have the generalized formula a - g - e - f - b - f - Pro - g based on the conservative substitution groups discussed above.

Sweet Peptides

Applications: As non-nutrient sweeteners for food, drink, desserts, candies, chewing gums and medicine. Helpful in both normal and low calorie diets for reducing calorie intake. Useful in making bitter medicine and pills more palatable. Suitable for persons with diabetes.

Mechanism: These peptides bind to receptors of sweet tasting papillae and induce a sweet sensation.

Dose: Typically, these peptides are 5000 to 10,000 times sweeter than sugar. In comparison, aspartame is only 160 times sweeter than sugar.

Administration: Oral

Modifications: Other structural constraints, particularly cyclization, may improve the heat stability of these peptides. Stabilization should increase the usefulness of these polypeptides in cooking.

1. Origin Type: **Thaumatin**
Structures:

- Ala-Pro-Ala-Lys-Leu-Lys-Ala-Pro-Gly (SW-T1) (SEQ ID NO: 70)
- Ala-Pro-Gly-Ser-Ser-Asn-Tyr-Arg-Val-Thr-Phe-Ala-Pro-Thr-Ala (SW-T2) (SEQ ID NO: 71)
- Gly-Pro-Thr-Glu-Tyr-Ser-Arg-Phe-Phe-Lys-Arg-Leu-Ala-Pro-Asp (SW-T3) (SEQ ID NO: 72)
- Asp-Lys-Pro-Thr-Thr-Val-Thr-Ala-Pro-Gly (SW-T4) (SEQ ID NO: 73)
- Asn-Val-Pro-Met-Asn-Phe-Ser-Pro-Thr-Thr (SW-T5) (SEQ ID NO: 74)

2. Origin Type: **Monellin**
Structures:

- Ile-Arg-Pro-Ala-Met-Lys-Lys-Thr-Ile-Tyr-Glu-Asn-Glu (SW-M1) (SEQ ID NO: 75)
- Arg-Pro-Arg-Lys-Leu-Leu-Arg-Phe-Asn-Gly-Pro-Val (SW-M2) (SEQ ID NO: 76)

3. Origin Type: **Mabinlin**
Structures:

- Gln-Pro-Arg-Arg-Pro-Ala-Leu-Arg-Gln-Pro-Ala (SW-MB1) (SEQ ID NO: 77)
- Ala-Pro-Asn-Gln-Leu-Arg-Gln-Val-Asp-Arg-Pro-Ala (SW-MB2) (SEQ ID NO: 78)
- Ile-Pro-Asn-Ile-Gly-Ala-Ala-Pro-Phe-Arg-Ala-Trp (SW-MB3) (SEQ ID NO: 79)
- Ile-His-Arg-Arg-Ala-Gln-Phe-Gly-Gly-Gln-Pro-Asp (SW-MB4) (SEQ ID NO: 80)
- Leu-Pro-Asn-Ile-Ala-Asn-Ile-Pro-Asn (SW-MB5) (SEQ ID NO: 81)

Taste-Modifier Peptides

Applications: As modifiers of sour taste into sweet taste.

Mechanism: Probably through interaction with taste receptors.

Dose: 30 nM to 500 nM.

Administration: Oral.

Modifications: Structural constraints, particularly cyclization of the peptides, may help in the heat stability of these peptides. Stabilization should increase the usefulness of these polypeptides in cooking.

1. Origin Type: **Miraculin**

Structures:

- Asp-Arg-Pro-Leu-Ala-Phe-Phe-Pro-Glu-Asn-Pro-Lys-Glu (TM-MIR1) (SEQ ID NO: 82)
- Thr-Thr-Pro-Asn-Gly-Thr-Phe-Val-Ala-Pro-Arg-Val (TM-MIR2) (SEQ ID NO: 83)

2. Origin Type: **Curculin**

Structure:

- Tyr-Gly-Pro-Val-Leu-Trp-Ser-Leu-Gly-Pro-Asn-Gly (TM-CUR1) (SEQ ID NO: 84)

Macrophage Activating Peptide

Origin Type: **Interferon gamma**

Applications: The following peptide should activate macrophages for tumor cytotoxicity and to kill parasites. It should be useful in treatment of malaria and other parasitic diseases.

Mechanism: Bind to specific receptors on macrophage surface.

Dose: 10-500 nM.

Administration: Intravenous injections, *in situ* injections, inhalation, oral administration using

coated polymers, dermal patches and other appropriate means.

Structure: Ser-Pro-Ala-Ala-Lys-Thr-Pro-Lys-Arg (IFNG1) (SEQ ID NO: 85)

Anti-contraction Peptides

Application: To prevent premature labor in pregnant women.

Origin Type: **Relaxin**

Mechanism: Bind to relaxin receptors and induce uterine relaxation.

Dose: 1-20 nmole/mouse

Administration: Intravenous injections, *in situ* injections, inhalation, oral administration using coated polymers, dermal patches and other appropriate means.

Structures:

- Leu-Pro-Gly-Arg-Glu-Leu-Val-Arg-Ala-Gln-Ile-Ala-Ile-Pro-Gly (ACP-R1) (SEQ ID NO: 86)
- Leu-Pro-Gly-Arg-Glu-Leu-Val-Arg-Ala-Val-Ile-Gln-Ile-Pro-Gly (ACP-RA) (SEQ ID NO: 87)

Homology: The ACP-R1 and ACP-RA polypeptides have the generalized formula b - Pro - g - a - d - b - b - a - g based on the conservative substitution groups discussed above.

Antitumor Peptides

Mechanism: Interact with specific receptors and inhibit tumor growth.

Dose: 0.5-10 nM.

Administration: Intravenous injections, *in situ* injections, inhalation, oral administration using coated polymers, dermal patches or other suitable means.

1. Origin Type: **Oncostatin M**

Structures:

- Asp-Pro-Tyr-Ile-Arg-Ile-Gln-Gly-Leu-Asp-Val-Pro-Lys-Leu (ATP-OSM1) (SEQ ID NO: 88)

- Glu-Arg-Pro-Gly-Ala-Phe-Pro-Ser-Glu (ATP-OSM2) (SEQ ID NO: 89)
- Glu-Pro-Thr-Lys-Ala-Gly-Arg-Gly-Ala-Ser-Gln-Pro-Ala (ATP-OSM3) (SEQ ID NO: 90)

2. Origin Type: **Leukemia Inhibitory Factor**
Structures:

- Thr-Pro-Val-Asn-Ala-Thr-Pro-Ala (ATP-LIF1) (SEQ ID NO: 91)
- Thr-Pro-Ala-Ile-Arg-His-Pro-Ala (ATP-LIF2) (SEQ ID NO: 92)
- Phe-Pro-Asn-Asn-Leu-Asp-Lys-Leu-Pro-Gly (ATP-LIF3) (SEQ ID NO: 93)
- Gly-Pro-Asn-Val-Thr-Asp-Phe-Pro-Ser (ATP-LIF4) (SEQ ID NO: 94)

Antiplatelet Peptides

1. Origin Type: **Leech Antiplatelet Protein**
Mechanism: Interacts with collagen receptor and inhibits collagen-induced platelet aggregation.
Dose: 1-4 μM.
Administration: Intravenous injections, *in situ* injections, local applications, inhalation, oral administration using coated polymers, dermal patches.
Structures:

- Lys-Arg-Pro-Gly-Trp-Lys-Leu-Pro-Asp-Asn (APCol-1) (SEQ ID NO: 95)
- Met-Pro-Glu-Glu-Ser-Ala-Val-Glu-Pro-Ser (APCol-2) (SEQ ID NO: 96)

2. Origin Type: **Moubatin**
Mechanism: Interacts with collagen receptor and inhibits collagen-induced platelet aggregation.
Dose: 1-4 μM.
Administration: Intravenous injections, *in situ* injections, local applications, inhalation, oral administration using coated polymers, dermal patches or other appropriate means.

Structures:

- Asp-Pro-Gln-Ala-Arg-Asp-Pro-Leu-Lys-Gly-Thr-Pro-Asn (APCol-M1) (SEQ ID NO: 97)
- Thr-Pro-Asn-Gly-Asn-Arg-Asp-Gly-Asn-Thr-Leu-Pro-Val (APCol-M2) (SEQ ID NO: 98)

Anti-fibrinogen peptides

Origin Type: **Monoclonal Antibody against the Fibrinogen α chain**

Mechanism: Interferes with fibrin polymerization

Dose: 1-2 mM.

Administration: Intravenous injections, *in situ* injections, local applications, inhalation, oral administration using coated polymers, dermal patches and other appropriate means.

Structure:

- His-Pro-Gly-Ile-Ala-Glu-Phe-Pro-Ser-Arg-Ala (AC-9E9) (SEQ ID NO: 99)

Alzheimer's Disease - HGIF

Applications: The brain of Alzheimer's disease patients contains reduced amounts of a growth inhibitory factor (GIF) which is abundant in normal human brain. This may account for the increased neurotrophic activity, leading to massive sprouting of cortical neurons, cell exhaustion and death. The following peptide should replace GIF and hence prevent the development of the disease.

Origin Type: **Human Growth Inhibitory Factor**

Dose: 1-5000 nM.

Administration: Intravenous injections, *in situ* injections, local applications, inhalation, oral administration using coated polymers, dermal patches and other appropriate means.

Structure:

- Ala-Pro-Ser-Gly-Gly-Ser-Pro-Thr (ADP-GIF1) (SEQ ID NO: 100)

EXAMPLE III. TRUNCATED ANALOGS AS ANTAGONISTS OR INHIBITORS

The following analogs can function as antagonists or inhibitors of natural polypeptides by binding to the natural polypeptide or by competing with the natural polypeptides for their interaction partner(s). These analogs are shorter than their natural counterparts and, thus, are truncated analogs.

Antifertility Peptides

Applications: In population control as a reversible antifertility measure.

Origin Type: **LHRH Receptor**

Dose: 5-5000 μM

Mechanism: Bind to gonadotropin releasing hormone and thus interfere with the fertility of men and women.

Administration: Intravenous injections, inhalation, oral administration using coated polymers, dermal patches and other appropriate means.

Structures:

- Ile-Pro-Leu-Met-Gln-Gly-Asn-Leu-Pro-Thr (AFP-LHRHR1) (SEQ ID NO: 101)
- Asp-Pro-Glu-Met-Leu-Asn-Arg-Leu-Ser-Asp-Pro-Val (AFP-LHRHR2) (SEQ ID NO: 102)
- Leu-Pro-Thr-Leu-Thr-Leu-Ser-Pro-Lys (AFP-LHRHR3) (SEQ ID NO: 103)

Anti-contraction Peptides

Application: To prevent premature labor in pregnant women.

Origin Type: **Angiotensin II Receptor (type 1)**

Mechanism: Interact with angiotensin II and abrogate its ability to induce contraction.

Dose: 1-200 μM.

Administration: Intravenous injections, *in situ* injections, inhalation, oral administration using coated polymers, dermal patches and other appropriate means.

Structures:

- Asp-Pro-Ile-Lys-Arg-Ile-Gln-Asp-Asp-Ala-Pro-Lys-Ala (ACP-AT1RA) (SEQ ID NO: 104)
- Val-Pro-Ala-Phe-His-Tyr-Glu-Ser-Gln-Asn-Ser-Thr-Leu-Pro-Ile (ACP-AT1RB) (SEQ ID NO: 105)
- Trp-Pro-Phe-Gly-Asn-Val-Leu-Pro-Lys (ACP-AT1RC) (SEQ ID NO: 106)

Anti-inflammatory Peptides

1. Origin Type: **Interleukin-8 receptor**

Mechanism: Bind to interleukin-8 and inhibit its ability to act as a chemo-attractant, and thus abrogate the pro-inflammatory effects of the interleukin.

Dose: 5-50 nM.

Administration: Intravenous injections, *in situ* injections, inhalation, oral administration using coated polymers, dermal patches and other appropriate means.

Structures:

- Leu-Pro-Pro-Phe-Leu-Leu-Asp-Ala-Ala-Pro-Ala (AIP-IL8R1) (SEQ ID NO: 107)
- Glu-Pro-Glu-Ser-Leu-Glu-Ile-Asn-Lys-Pro-Tyr (AIP-IL8R2) (SEQ ID NO: 108)

2. Origin Type: **Macrophage migration inhibitors**

Mechanism: Interacts with specific receptors and inhibits the migration of macrophages, thus stopping pro-inflammatory response.

Dose: 5-100 nM.

Administration: Intravenous injections, *in situ* injections, inhalation, oral administration using coated polymers, dermal patches and other appropriate means.

Structure:

- Lys-Pro-Pro-Gln-Tyr-Ile-Ala-Val-His-Val-Val-Pro-Asp-Gln (AIP-MIF1) (SEQ ID NO: 109)

3. Origin Type: **Fibrinogen γ-chain**

Mechanism: Inhibits the interactions between fibrinogen and its leukocyte receptor CD11b/CD18 integrin (Mac-1).

Dose: 0.8-20 μM.

Administration: Intravenous injections, *in situ* injections, inhalation, oral administration using coated polymers, dermal patches and other appropriate means.

Structure:

- Asn-Pro-Trp-Thr-Val-Phe-Gln-Lys-Arg-Leu-Asp-Pro-Ser-Val (AIP-FBG1) (SEQ ID NO: 110)

Platelet Derived Growth Factor Inhibitors

Origin Type: **Platelet-Derived Growth Factor**

Mechanism: Blocks binding of platelet-derived growth factor (PDGF) to its receptor, which blocks the effects of PDGF on smooth muscle.

Dose: 5-5000 μM.

Administration: Intravenous injections, *in situ* injections, inhalation, oral administration using coated polymers, dermal patches and other appropriate means.

Structures:

- Pro-Ser-Gly-Ser-Ala-Pro (PGF-1) (SEQ ID NO: 111)
- Pro-Arg-Val-Thr-Asp-Pro (PGF-2) (SEQ ID NO: 112)
- Pro-Arg-Gly-Arg-Gly-Met-Pro-Gln-Pro (PGF-3) (SEQ ID NO: 113)

Blood Protein Inhibitors and Antagonists

1. Origin Type: **Factor V**

Structures:

- Glu-Met-Lys-Ala-Ser-Lys-Pro-Gly-Trp-Trp-Leu (AC-5A1) (SEQ ID NO: 114)
- Leu-Pro-Gly-Ser-Phe-Lys-Thr-Leu-Glu-Met-Lys-Ala-Ser-Lys-Pro-Gly (AC-5A2) (SEQ ID NO: 115)

Mechanism: Interferes with the generation of thrombin from prothrombin.

Dose: 1-2 mM

Administration: Intravenous injections, *in situ* injections, local applications, inhalation, oral administration using coated polymers, dermal patches and other appropriate means.

2. Origin Type: **Factor VIII**

Mechanism: Interferes with the activation of factor X

Dose: 1-2 mM

Administration: Intravenous injections, *in situ* injections, local applications, inhalation, oral administration using coated polymers, dermal patches and other appropriate means.

Structures:

- Glu-Met-Leu-Pro-Ser-Lys-Ala-Gly-Ile-Trp-Arg (AC-8A1) (SEQ ID NO: 116)
- Tyr-Pro-Gly-Val-Phe-Glu-Thr-Val-Glu-Met-Leu-Pro-Ser (AC-8A2) (SEQ ID NO: 117)

3. Origin Type: ***Naja nigricollis*** **phospholipase CM-IV**

Mechanism: Interferes with coagulation

Dose: 1-2 mM

Administration: Intravenous injections, *in situ* injections, local applications, inhalation, oral administration using coated polymers, dermal patches and other appropriate means.

Structures:

- Tyr-Glu-Lys-Ala-Gly-Lys-Met-Gly-Ala-Trp-Pro-Tyr (AC-PL1) (SEQ ID NO: 118)
- Trp-Pro-Tyr-Leu-Thr-Leu-Tyr-Lys-Tyr-Lys-Ala-Ser-Ala (AC-PL2) (SEQ ID NO: 119)

4. Origin Type: **Prothrombin**

Mechanism: The native polypeptide, also known as factor II, is the precursor of thrombin. The truncated analog binds with factors that would otherwise generate thrombin from prothrombin.

Dose: 50-5000 μM.

Administration: Intravenous injections, *in situ* injections, local applications, inhalation, oral administration using coated polymers, dermal patches and other appropriate means.

Structures:

- Ser-Pro-Trp-Gln-Val-Met-Leu-Phe-Arg-Lys-Ser-Pro-Gln-Glu-Leu-Leu-Pro-Gly (ACS-THR1) (SEQ ID NO: 120)
- Leu-Pro-Arg-Lys-Ser-Pro-Gln-Glu-Leu-Leu-Pro-Gly (ACS-THR2) (SEQ ID NO: 121)
- Ile-Pro-Lys-His-Ser-Arg-Thr-Arg-Tyr-Pro-Arg-Asn-Ile-Glu-Lys (ACS-THR3) (SEQ ID NO: 122)

Homology: The ACS-THR1 and ACS-THR2 polypeptides have the generalized formula a - a - f - Pro - e - d - b - b - Pro - g based on the conservative substitution groups discussed above.

5. Origin Type: **Factor Xa**

Mechanism: Interferes in the generation of thrombin from prothrombin.

Dose: 50-5000 μM.

Administration: Intravenous injections, *in situ* injections, local applications, inhalation, oral administration using coated polymers, dermal patches or other appropriate means.

Structures:

- Ala-Pro-Trp-Gln-Ala-Leu-Leu-Ile-Asn-Glu-Glu-Asn-Glu-Gly-Phe-Pro-Gly (ACS-Xa1) (SEQ ID NO: 123)
- Leu-Pro-Asn-Glu-Glu-Asn-Glu-Gly-Phe-Pro-Gly (ACS-Xa2) (SEQ ID NO: 124)
- Leu-Pro-Asn-Glu-Glu-Asn-Glu-Pro-Phe (ACS-Xa3) (SEQ ID NO: 125)
- Val-Pro-Asp-Arg-Asn-Thr-Glu-Gln-Glu-Glu-Pro-Gly (ACS-Xa4) (SEQ ID NO: 126)

Homology: The ACS-Xa2 and ACS-Xa3 polypeptides have the generalized formula b - Pro - e - d - d - e -

d based on the conservative substitution groups discussed above.

6. Origin Type: **Factor IXa**

Mechanism: Interferes with the activation of factor X

Dose: 50-5000 μM.

Administration: Intravenous injections, *in situ* injections, local applications, inhalation, oral administration using coated polymers, dermal patches and other appropriate means.

Structure:

- Phe-Pro-Trp-Gln-Val-Val-Leu-Asn-Gly-Lys-Val-Asp-Ala-Phe-Pro-Gly (ACS-IXa1) (SEQ ID NO: 127)
- Asn-Pro-Lys-Val-Asp-Ala-Phe-Pro-Gly (ACS-IXa2) (SEQ ID NO: 128)
- Ala-Pro-Glu-His-Asn-Ile-Glu-Glu-Thr-Glu-His-Thr-Glu-Pro-Lys (ACS-IXa3) (SEQ ID NO: 129)

7. Origin Type: **Factor VIIa**

Mechanism: Interferes with the activation of factor X

Dose: 50-5000 μM.

Administration: Intravenous injections, *in situ* injections, local applications, inhalation, oral administration using coated polymers, dermal patches and other appropriate means.

Structures:

- Ala-Pro-Trp-Gln-Val-Leu-Leu-Leu-Val-Asn-Gly-Ala-Gln-Leu-Pro-Gly (ACS-VIIa1) (SEQ ID NO: 130)
- Ala-Pro-Trp-Gln-Val-Leu-Leu-Leu-Val-Asn-Pro-Ala-Gln-Leu-Pro-Gly (ACS-VIIa2) (SEQ ID NO: 131)
- Leu-Pro-Glu-His-Asp-Leu-Ser-Glu-His-Asp-Pro-Asp (ACS-VIIa3) (SEQ ID NO: 132)

Homology: The ACS-VIIa1 and ACS-VIIa2 polypeptides have the generalized formula g - Pro - c - e - b - b - b - b - b - e based on the conservative substitution groups discussed above.

Antiplatelet Peptides

Several blood proteins are useful for their antiplatelet properties. The proteins can be used as antithrombotic drugs for the prevention and treatment of myocardial infarction, stroke and other related disorders. These proteins may have significant antitumor effects, as well as being useful for wound healing.

1. Origin Type: **von Willebrand Factor**

Structure: Ala-Pro-Leu-His-Asp-Phe-Tyr-Pro-Ser (AAP-VWF1) (SEQ ID NO: 133)

Mechanism: Interferes in the interaction between von Willebrand factor and glycoprotein Ib and thus inhibits platelet agglutination.

Dose: 10-50 μM.

Administration: Intravenous injections, *in situ* injections, local applications, inhalation, oral administration using coated polymers, dermal patches and other appropriate means.

2. Origin Type: **Platelet Glycoprotein IIb**

Structure: Gln-Pro-Asn-Asp-Gly-Gln-Pro-His (AAP-GPIIb1) (SEQ ID NO: 134)

Mechanism: Interferes in the interaction of glycoprotein IIb with adhesive ligands.

Dose: 5-100 μM.

Administration: Intravenous injections, *in situ* injections, local applications, inhalation, oral administration using coated polymers, dermal patches and other appropriate means.

EXAMPLE IV. HOMOLOGS

Polypeptide homologs can be based upon biologically active polypeptides, such as naturally-occurring polypeptides or polypeptides derived therefrom, which do not contain conformation-constraining moieties, such as proline, around

interaction sites. Such polypeptides can be altered by inserting conformation-constraining moieties into the polypeptide so that these moieties bracket the interaction site.

Homologs of polypeptides that already contain conformation-constraining brackets also can be made, according to the present invention, by altering the location or structure of the bracket. For instance, a naturally-occurring proline residue that is within five amino acids of an interaction site can be moved to be within two amino acids of the interaction site. Additionally, a cyclic constraining moiety can be substituted with a proline to alter the properties of the interaction site. Furthermore, as stated above a homolog can also be shortened so that its length is less than that of the native polypeptide. These and other changes will become apparent in view of the teachings of this application.

Like the analogs, homologs can mimic the activity of the native polypeptide or serve as antagonists. The non-limiting examples below include mimicking and antagonizing homologs. The sequences of the native polypeptides are known.

Analgesics

Origin Type: **Enkephalins**

Application: Alleviation of pain and emotional stress.

Dose: 5-5000 μM.

Administration: Intravenous injections, *in situ* injections, inhalation, oral administration with coated polymers, dermal patches, and other appropriate means.

Structures:

- Pro-Tyr-Gly-Gly-Phe-Met-Pro (AN-1) (SEQ ID NO: 135)
- Pro-Tyr-Gly-Gly-Phe-Leu-Pro (AN-2) (SEQ ID NO: 136)

Appetite Suppressant

Origin Type: **Cholecystokinin**

Application: Suppression of appetite.

Administration: Intravenous injections, *in situ* injections, inhalation, oral administration with coated polymers, dermal patches, and other appropriate means.

Dose: 5-5000 μM.

Structure:

- Pro-Phe(4-tetrazole)-Met-Gly-Trp-Met-Asp-Phe-Pro (AS-1) (SEQ ID NO: 137)

B-Cell Differentiating Peptide

Origin Type: **B-Cell Differentiating Peptide**

Application: Treatment of immune disorders resulting from low levels of gamma globulin

Administration: Intravenous injections, *in situ* injections, inhalation, oral administration with coated polymers, dermal patches, and other appropriate means.

Dose: 5-5000 μM.

Structure:

- Pro-Lys-His-Gly-Pro (BCD-1) (SEQ ID NO: 138)

Hypocalcemic Agent

Origin Type: **Calcitonin**

Application: Mimics human calcitonin. Lowers blood calcium and phosphate levels, and prevents demineralization of bones.

Administration: Intravenous injections, *in situ* injections, inhalation, oral administration with coated polymers, dermal patches, and other appropriate means.

Dose: 5-5000 μM.

Structure:

- Pro-Gln-Thr-Ala-Ile-Gly-Val-Gly-Ala-Pro (HCA-1) (SEQ ID NO: 139)

Hypoglycemic Potentiator

Origin Type: **Human Growth Hormone**

Application: Useful for lowering the effective dosages of insulin.

Administration: Intravenous injections, *in situ* injections, inhalation, oral administration with coated polymers, dermal patches, and other appropriate means.

Dose: 5-5000 μM.

Structure:

- Pro-Glu-Glu-Ala-Tyr-Ile-Pro-Lys (HGP-1) (SEQ ID NO: 140)

Hypotensive Agent

Origin Type: **Prolyl-phenylalanyl-arginine chains**

Application: Reduction of blood pressure by reducing kidney vessel resistance.

Administration: Intravenous injections, *in situ* injections, inhalation, oral administration with coated polymers, dermal patches, and other appropriate means.

Dose: 5-5000 μM.

Structure:

- Pro-Pro-Phe-Arg-Pro (HTA-1) (SEQ ID NO: 141)

Immune Potentiator

Application: Stimulates differentiation of stem cells into thymus-derived cells and antibody production.

Administration: Intravenous injections, *in situ* injections, inhalation, oral administration with coated polymers, dermal patches, and other appropriate means.

Dose: 5-5000 μM.

1. Origin Type: **Thymopoietin**

   Structure:

   - Arg-Pro-Asp-Gly-Trp-Pro (IP-1) (SEQ ID NO: 142)

2. Origin Type: **Thymosin α1**

Structure:

- Pro-Val-Glu-Glu-Ala-Glu-Asn-Pro (IP-2) (SEQ ID NO: 143)

Somatostatin-like Peptide

Origin Type: **Somatostatin**

Application: To inhibit oversecretion of glucagon and/or growth hormone in conditions such as acromegaly and diabetes.

Administration: Intravenous injections, *in situ* injections, inhalation, oral administration with coated polymers, dermal patches, and other appropriate means.

Dose: 5-5000 μM.

Structure:

- Pro-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Pro (SLP-1) (SEQ ID NO: 144)

Gastrin-releasing Peptide Antagonists

Origin Type: **Gastrin Releasing Peptide**

Application: Inhibits the action of gastrin-releasing peptide. Can be used for treatment of small cell lung carcinoma by prevention of the growth-promoting action of gastrin-releasing peptide.

Administration: Intravenous injections, *in situ* injections, inhalation, oral administration with coated polymers, dermal patches, and other appropriate means.

Dose: 5-5000 μM.

Structure:

- Pro-His-Trp-Ala-Val-Gly-His-Leu-Pro (GRP-1) (SEQ ID NO: 145)

The foregoing description, specific examples and data, while indicating preferred embodiments, are given by way of illustration and are not intended to limit the present invention. Various changes and modifications within the present invention will be apparent to the skilled artisan from the discussion and disclosure contained herein.

WHAT IS CLAIMED IS:

1. An analog of a biologically-active polypeptide, comprising an interaction site and conformation-constraining moieties flanking said interaction site.

2. An analog according to claim 1, wherein said analog has no more than 30 amino acid residues.

3. An analog according to claim 1, wherein said analog is a truncated analog.

4. An analog according to claim 1, wherein said conformation-constraining moieties are within seven amino acid residues of said interaction site.

5. An analog according to claim 1, wherein said conformation-constraining moieties are proline residues.

6. An analog according to claim 1, wherein said analog mimics an activity of said biologically-active polypeptide.

7. An analog according to claim 6, wherein said biologically-active polypeptide is selected from the group consisting of hypotensive peptides, fibrinolytic peptides, chemotactic peptides, growth promoter peptides, lymphocyte mitogens, immunomodulator peptides, clot-inducing peptides, cardiac stimulant peptides, sweet peptides, taste-modifier peptides, macrophage activating peptides, anti-tumor peptides, Relaxin, platelet aggregation inhibitors and Alzeimer's disease peptides.

8. An analog according to claim 1, wherein said analog inhibits or antagonizes an activity of said biologically-active polypeptide.

9. An analog according to claim 8, wherein said biologically-active polypeptide is selected from the group consisting of fertility peptides, inflammatory peptides, platelet derived growth factors, blood proteins, and Angiotensin II.

10. An analog according to claim 9, wherein said blood protein is selected from the group consisting of Factor V, Factor VIIa, Factor VIII, Factor IXa, Factor Xa, fibrinogen, von Willebrand Factor, Platelet Glycoprotein IIb and prothrombin.

11. A homolog of a biologically-active polypeptide having an interaction site, wherein said homolog comprises a conformation-constraining moiety placed proximate to said interaction site.

12. A homolog according to claim 11, wherein said conformation-constraining moiety is a proline residue.

13. A homolog according to claim 11, wherein said homolog mimics an activity of said biologically-active polypeptide.

14. A homolog according to claim 13, wherein said biologically-active polypeptide is selected from the group consisting of analgesics, appetite suppressants, B-cell differentiating peptides, hypocalcemic agents, hypoglycemic potentiators, hypotensive agents, immune potentiators and somatostatin-like peptides.

15. A homolog according to claim 11, wherein said homolog inhibits or antagonizes an activity of said biologically-active polypeptide.

16. A homolog according to claim 17, wherein said naturally-occurring polypeptide is a gastrin-releasing peptide.

17. A composition comprising an analog according to claim 1 and a pharmaceutically acceptable carrier.

18. A composition comprising a homolog according to claim 11 and a pharmaceutically acceptable carrier.

19. A method for providing an analog of a biologically-active polypeptide, comprising the steps of:

identifying an interaction site of a biologically-active polypeptide, and

obtaining an analog that (i) has a different length than said biologically-active polypeptide and (ii) contains said interaction site of said biologically-active polypeptide, wherein said interaction site is flanked by conformation-constraining moieties.

20. A method for providing an analog according to claim 19, wherein said analog is no more than 30 amino acid residues in length.

21. A method for providing an analog according to claim 19, wherein said conformation-constraining moieties are proline residues.

22. A method according to claim 19, wherein said conformation-constraining moieties are proline residues.

23. A method according to claim 19, wherein said obtaining is performed by a polymerase chain reaction or by peptide synthesis.

24. A method for providing a homolog of a biologically-active polypeptide, comprising the steps of:

identifying an interaction site of said biologically-active polypeptide and

flanking said interaction site of said biologically-active polypeptide with conformation constraining moieties.

25. A method according to claim 24, wherein said conformation-constraining moieties are proline residues.

26. A method according to claim 24, wherein said conformation-constraining moieties are cysteine residues.

27. A method according to claim 24, wherein said flanking step is performed by a polymerase chain reaction or peptide synthesis.

28. An analog of a biologically-active polypeptide obtainable by a process according to claim 19.

29. A homolog of a biologically-active polypeptide obtainable by a process according to claim 24.

30. A method of identifying an interaction site of a polypeptide, comprising the step of searching a polypeptide for the presence of sites that are flanked by conformation-constraining moieties.

31. A method according to claim 31, wherein said conformation-constraining moieties are proline residues or cysteine residues.

INTERNATIONAL SEARCH REPORT

Internati Application No
PCT/US 94/04294

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C07K7/06 A61K37/02 C07K3/08 C07K1/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	N YOSHIAKI & T SEKIGUCHI 'protein stability and stabilization through protein engineering' 1991 , ELLIS HORWOOD , NEW YORK (USA) see paragraph 6.2.1.2, page 157 ---	1-31
X	PEPTIDES. CHEMISTRY AND BIOLOGY. PROCEEDINGS OF THE 12TH AMERICASN PEPTIDE SYMPOSIUM, JUNE 16-21, CAMBRIDGE, MASS., 1991, ESCOM, LEIDEN (THE NETHERLANDS) pages 378 - 380 E KITAYUNI ET AL. 'Design of alpha-helical coiled coil peptide containing periodic proline residues' see the whole document --- -/--	1-31

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

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"&" document member of the same patent family

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Name and mailing address of the ISA
European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax (+31-70) 340-3016

Authorized officer
Masturzo, P

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA., vol.82, no.23, December 1985, WASHINGTON US pages 8057 - 8061 E F PLOW ET AL. 'The effect of Arg-Gly-Asp-containing peptides on fibrinogen and von Willebrand factor binding to platelets' see table 1 ---	7
X	WO,A,91 11458 (GENENTECH) 8 August 1991 see the whole document -----	7

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 94/04294

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9111458	08-08-91	NONE	